



UNIVERSITY OF
TECHNOLOGY SYDNEY

The Uptake of Drugs by Necrophagous Insects

by

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Certificate of Authorship/ Originality

I certify that the work in this thesis has not been submitted for a degree or as part of the requirements for a degree.

To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

A handwritten signature in dark ink, appearing to read 'Daniel Kenny', with a long, sweeping horizontal stroke extending to the right.

Daniel Kenny

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Abstract

Insects have been used for several decades around the world to assist forensic scientists and pathologists in determining the time elapsed since death, by analysing their growth. Whilst the rate of larval growth is primarily affected by temperature, entomologists soon discovered that the presence of certain drugs can also significantly accelerate or decelerate larval growth rates. It was from this research that scientists discovered the ability of fly larvae to accumulate drugs within their body and toxicologists became interested in the possibility of exploiting insects feeding upon the decaying tissues of a human body as alternative toxicological specimens. Theoretically, insects offered great potential over the 'traditional' specimens (blood, urine, organs), which are degraded by the decomposition process and become extremely difficult to analyse. Consequently research into the field of entomotoxicology began and soon divided the toxicological community. Some academics argued that their results from larval analysis displayed enormous variance and bore no relationship to cadaver concentrations whilst others maintained that under controlled study correlations between larval and body concentrations have been found.

The primary objective of this research was to determine what effect a variety of variables common to cadaver ecology would have on larval drug uptake. While previous studies have employed traditional methods of analysis with GC-MS and HPLC, for these experiments a new method of analysis was devised with LC-MS-MS which offered vast improvements in processing time and adaptability. A suitable method for the

preservation of larval samples for the purpose of drug analysis was also devised. Larvae samples were exposed to a range of amitriptyline concentrations and then killed and preserved either by freezing or storage in a 70% ethanol solution after a brief immersion in boiling water. The analysis of amitriptyline concentration in larval samples tested at various times over a 12 month period demonstrated that freezing was the superior method of larval storage for drug analysis.

A number of experiments were then designed to determine the effect that variables such as temperature, larval activity, larval species, drug dose and the presence of multiple substances have on the larval uptake of drugs. These progressively relaxed the controls on the feeding environment so that each effect could be determined separately. Thus initial experiments compared larval uptake of amitriptyline in three constant temperatures. Next the impact of fluctuating temperature and larval species on the uptake of amitriptyline was determined. Finally larvae were exposed to amitriptyline and caffeine to determine the impact of a second substance (caffeine) on the larval uptake of the primary drug (amitriptyline).

These experiments have conclusively shown that larval drug uptake is significantly affected by these variables. Decreasing temperature causes a significant decrease in the rate and level of larval drug uptake. Fluctuating temperature compared to constant temperature has a similar effect. When a second substance was introduced again the amitriptyline concentration in the larval samples dropped. Despite this, larval drug concentrations were found to correlate linearly with their foodstuff concentrations (i.e. as

the drug concentrations in their food increased the drug concentrations in the larval also increased in linear proportions). However this was limited to larvae that were actively feeding.

Insects (especially blowfly larvae) that associate with human remains can be a valuable resource in forensic toxicology. However the fact that drug concentrations are significantly affected by temperature and the nature of the drugs present means that these must be considered when attempting to estimate the original cadaver drug concentrations from the larval concentrations.

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List of Abbreviations

Ami	Amitriptyline
[Ami]	Amitriptyline concentration
Caff	Caffeine
[Caff]	Caffeine concentration
ECD	Electron capture detector
DAD	Diode array detector
e.g.	For example
ESI	Electro-spray ionisation
FDA	Food and Drug Administration
FID	Flame Ionisation Detector
FPD	Flame photometric
g	Grams
GC	Gas chromatography
hrs	Hours
HPLC	High performance liquid chromatography
Ka	acid dissociation constant
kg	Kilograms
L	Litre
LC	Liquid chromatography
LD50	Dose that kills 50% of test subjects
LLE	Liquid-liquid extraction

LOQ	Limit of quantification
LLOQ	Lower limit of quantification
LOD	Limit of detection
m	metre
M	Moles
mg	Milligrams
min	Minutes
MISPE	Molecularly Imprinted Solid Phase Extraction
mL	Millilitres
mm	Millimetres
MS	Mass spectrometry
MS-MS	Tandem mass spectrometry
m/z	Mass to charge ratio
n	Number of samples or tests
n.a	Not applicable
n.d	Not detected
NMR	Nuclear magnetic resonance
NPD	Nitrogen phosphorous detector
p	Probability ratio
PFPA	Pentafluoropropionic acid
pKa	logarithmic measure of the acid dissociation constant
pg	picogram
pH	cologarithm of the activity of dissolved hydrogen ions

PMI	Post mortem interval
ppm	Parts per million
ng	Nanograms
μL	Microlitres
R	Correlation coefficient
rpm	Revolutions per minute
s	Seconds
SIM	Selected ion monitoring
SLM	Supported liquid membrane
SPE	Solid-phase extraction
SPME	Solid-phase micro extraction
spp	Species
TCD	Thermal conductivity detector
TLC	Thin layer chromatography
TMEDA	Tetramethylethylenediamine
UV	Ultraviolet
V	Volts
~	approximately
°C	Celsius
μg	Micrograms
%	Percent

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